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Derek P. Freyberg
Derek P. Freyberg, Reg. No. 29,250

4/8/99

Date

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

John J. Nestor et al.

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App. No.: 08/453,223

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Art Unit: 1611

Filed: May 30, 1995

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Examiner: Mark L. Berch

For: 2-(2-AMINO-1,6-DIHYDRO-6-OXO-PURIN-9-YL)METHOXY-
1,3-PROPANEDIOL DERIVATIVE

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

DECLARATION OF CHARLES DVORAK

I, Charles Dvorak, a citizen of the United States residing in Palo Alto, California, declare as follows:

I received a BS in Chemistry with Honors from Humboldt State University, Arcata, California, in 1969. I have been employed as a chemist at the Syntex Research division of Syntex (U.S.A.) Inc., now Roche Bioscience, since October 1969, as a process chemist, and am now a Principal Scientist at Roche Bioscience. I have worked extensively in the synthesis of ganciclovir and its esters and related nucleosides; in particular in the development of crystalline ganciclovir monovalinate hydrochloride, and processes for its preparation and purification.

I have reviewed and am familiar with US Patent No. 5,043,339 to Lilia M. Beauchamp (the "Beauchamp patent"), cited as a reference against this application.

As a part of the development of ganciclovir mono(L-valinate) as a pharmaceutical agent, I have been involved in the development of a crystalline salt of ganciclovir mono(L-valinate) that would desirably be easily preparable and stable.

Based on:

- (1) the inability of myself and others to prepare crystalline ganciclovir mono(L-valinate) acetate,
- (2) the ready ability to prepare crystalline ganciclovir mono(L-valinate) hydrochloride, and
- (3) the inability of others to prepare crystalline ganciclovir bis(L-valinate) acetate, for reasons which I discuss below in my **Conclusions**, I determined to attempt the preparation of ganciclovir bis(L-valinate) hydrochloride, believing that it would be substantially more likely to be preparable in crystalline form than the corresponding acetate salt prepared in the Beauchamp patent.

I, or persons working with me, have conducted the following experiments in an attempt to prepare crystalline ganciclovir bis(L-valinate) hydrochloride (GBVH).

**Preparation of ganciclovir bis(CBZ-L-valinate)
[following Example 5(a) of the Beauchamp patent]**

A solution of 22.5 g *N*-benzyloxycarbonyl-L-valine (CBZ-L-valine), 18.6 g *N,N*-dicyclohexylcarbodiimide, and 1.2 g 4-dimethylaminopyridine were stirred in 100 mL dimethylformamide under nitrogen for 10 minutes. 7.65 g ganciclovir were added in 20 mL dimethylformamide, and the mixture was stirred for 18 hours at ambient temperature.

The resulting suspension was filtered, and the filter cake was washed with dichloromethane. The filtrate was stripped to yield a very viscous oil (yield 32 g). The crude product was

spotted on a silica TLC plate, co-spotted with ganciclovir, and ganciclovir spotted as the reference. The plate was eluted in a 10% methanol/dichloromethane bath. The TLC chromatogram indicated a major apolar product ($R_f \sim 0.9$) and a more polar impurity ($R_f \sim 0.3$), with no apparent ganciclovir.

The major apolar product, ganciclovir bis(CBZ-L-valinate), was purified as follows: after standing, the oily residue had solidified as a glass, which was dissolved in 100 mL methanol and the solution added to 65 g silica gel. The mixture was stirred briefly and then the solvent stripped to yield a sticky powder which was slurried with dichloromethane. The slurry was poured into a chromatography column into which a slurry of silica gel in dichloromethane had been previously poured and the silica gel allowed to settle. After the solid had settled, the supernatant dichloromethane was allowed to elute through the column. The column was then eluted with a methanol/dichloromethane mixture, which was initially 1% methanol. The percentage of methanol was increased incrementally as the chromatography progressed. Fractions collected were analyzed by TLC using the same conditions described above, comparing with a sample of the original methanol solution retained as a reference. Fractions containing the desired product were combined and stripped to yield 12.6 grams of a glass that was identified as ganciclovir bis(CBZ-L-valinate) by NMR. The NMR showed two multiplets for the valine isopropyl methyl groups, and broad multiplets for the valine methyne, and the glycerol methylenes and methyne, with a sharp multiplet for the aromatic protons and a multiplet for the glycosidic methylene.

Preparation of GBVH

[following Example 5(b) of the Beauchamp patent, except for using hydrochloric acid instead of acetic acid]

(1) A mixture of 0.23 g 20% $\text{Pd}(\text{OH})_2$ on carbon and 5 mL methanol was hydrogenated under ambient conditions overnight. 2.3 g ganciclovir bis(CBZ-L-valinate) from the preceding preparation was dissolved in 20 mL methanol and 0.65 g 37% hydrochloric acid was added with stirring. The solution was added to the

hydrogenation flask by syringe. The hydrogenation was continued at ambient temperature. Every hour, for the first 8 hours, the hydrogenation flask was vented to remove carbon dioxide that was generated. The hydrogenation was allowed to proceed for 64 hours. TLC indicated one baseline product. After the hydrogenation, the mixture was filtered and the filtrate stripped to afford a viscous oil. The oil was slowly diluted with isopropanol until the mixture became hazy. After stirring for about 2 hours, a sticky white solid had separated. After four hours of continued stirring, there was no further change and the mixture was diluted to a volume of 40 mL with isopropanol. More sticky solid separated, and the mixture was stirred vigorously overnight.

The sticky solid had hardened after stirring overnight. It was broken up and stirred in the mixture for 2 more hours. The solid was filtered, washed with cold isopropanol and dried in air. The product was deliquescent and drying was discontinued. It was dissolved in 1.5 grams water and the solution was added dropwise to 15 mL isopropanol. A small amount of solid separated. The mixture was diluted with 15 mL isopropanol and stirred overnight. The precipitation of solid seemed dependent on the isopropanol addition, each drop caused a fine cloudiness. The fine particles apparently clumped with the larger particles already present with continued stirring. If the dilution was stopped, the mixture did not apparently thicken as is usually the case with a standard crystallization (where crystals of the product grow in size and increase in number, thickening the mixture).

The precipitated solid was collected, washed with cold isopropanol, and was dried at about 35°C (yield 0.9 g). Not all solid washed from the walls of the precipitation flask and stuck to a spatula when scraped off the walls. The dried cake was a dull white with no crystalline sparkle or luster, indicating a lack of crystallinity, and the cake was not frangible. The material was very hygroscopic and HPLC indicated the product to consist of 60% of main product with two impurities (each 18%). HPLC method: Zorbax SB-C18 150 x 4.6 mm, 3.5 µm packing; 90%

100mM $(\text{NH}_4)\text{H}_2\text{PO}_4$ pH3/10% methanol solvent; 1mL/min isocratic elution at ambient temperature using a 254 nm UV detector. An NMR spectrum was consistent with the desired product ganciclovir bis(L-valinate) hydrochloride and showed that the material contained isopropanol. NMR showed a single multiplet for the valine isopropyl methyls, broad multiplets for the valine isopropyl methyne, the glycerol methylenes and methyne, and the valine methyne (superposed on a sharp multiplet for the isopropanol methyne), and a sharp doublet for the isopropanol methyls.

(2) The preceding hydrogenolysis was repeated with the following modifications:

0.4 g 20% $\text{Pd}(\text{OH})_2$ on carbon was pre-reduced as in (1) above. The hydrogenation of 4.1 g ganciclovir bis(CBZ-L-valinate) with 50 mL methanol and 1.1 g 37% HCl was monitored hourly by TLC. TLC indicated a mixture of products, of which one product was identical to the main product identified in (1) above and one product was of an intermediate R_f (~0.3) The amount of starting material gradually decreased and could no longer be detected after 4 hours. The amount of intermediate R_f product, which I believe to be the monobenzyloxycarbonyl intermediate, decreased and after 8 hours was almost gone. The reaction was allowed to proceed for 16 hours.

The initial workup was as described in (1) above. A sample of the sticky white solid was analyzed by HPLC using the conditions described above and showed 62% of the major product with two impurities, 18% each, essentially identical to the result in (1) above. The mother liquor showed a similar composition: 61.4% of the major product with 18.7% and 18% of the impurities. After dilution with isopropanol, the supernatant was decanted and the white sticky solid stirred with 40 mL fresh isopropanol. After isopropanol treatment, the solid had hardened and was collected, washed and dried (yield 2.6 grams). The appearance of the solid indicated a lack of crystallinity.

(3) The catalyst pre-reduction and the hydrogenation itself was carried out as in (1) above with the following modifications:

4.1 g ganciclovir bis(CBZ-L-valinate) was hydrogenated with 0.4 g 20% Pd(OH)₂ on carbon in 50 mL methanol and 1.1 g 37% HCl. After 45 minutes, TLC indicated starting material, the desired product, and the intermediate R_f product seen in (2) above. HPLC (conditions as above) of the same sample indicated a ratio of 62.2% main product and 17.7% and 19.1%, respectively of the two impurities seen in (2) above. The hydrogenation was continued for 72 hours. The mixture was filtered and the filtrate was stripped to obtain a glass. The residue was dissolved in 5 mL methanol and the solution was added slowly dropwise to 80 mL ethyl acetate with vigorous stirring. A solid precipitated and stuck to the walls of the glass. The solid hardened and was broken up with continued stirring. Stirring was continued further for 168 hours.

All the solid material stuck to the wall was broken up into a suspended solid. The mixture was allowed to stand. A small amount of solid was dried. The dried, white solid lacked luster, indicating a lack of crystallinity, and was hygroscopic. The product cake was quite hard to break up. The remaining product mixture was not filtered due to the poor quality of the sample. NMR was essentially identical to that for the material prepared in (1) above except for the absence of the peaks due to isopropanol.

Preparation of GBVH from ganciclovir bis(L-valinate) acetate

Ganciclovir bis(L-valinate) acetate was treated with hydrochloric acid to convert it into the hydrochloride salt as follows: ganciclovir bis(L-valinate) acetate was prepared by methods comparable to those described above for the hydrochloride; and HPLC of the ganciclovir bis(L-valinate) acetate starting material indicated 92% purity and 3.6% and 3.8% of the same impurities as found in the hydrogenated products of Experiments 1, 2, and 3.

0.5 g ganciclovir bis(L-valinate) acetate was added to a mixture of water and HCl (0.5 g water and 0.18 g 37% HCl) and stirred. After all solids dissolved, the solution was slowly diluted with isopropanol until it became cloudy. After stirring for one hour, a white, very sticky solid had separated. After 4 hours the solid was still sticky and various attempts to scratch the solid to obtain crystalline material failed. While the scratching experiments failed to induce crystallization, treatment with ethyl acetate appeared to harden the sticky solid. The mixture was diluted to 15 mL slowly with ethyl acetate and stirred for 16 hours.

All the solid was stuck to the walls of the flask and was still somewhat sticky. The supernatant was decanted and 5 mL isopropanol were added. The mixture was stirred and the solid hardened and was scraped periodically off the walls of the flask. Stirring was continued for 64 hours. The remaining solid material stuck to the bottom of the flask was scraped loose and stirring was continued for 16 hours. Thereafter the solid was collected, washed with isopropanol and dried at 45°C under nitrogen bleed, with a yield of 0.4 g ganciclovir bis(L-valinate) hydrochloride obtained. HPLC indicated 91.9% of the product with 3.62% and 3.68% of the two impurities seen in the previous preparations. A crystallinity study by powder X-ray diffraction indicated an amorphous material. The dried white solid lacked luster and was hygroscopic. The product cake was hard to break up, the material beginning to get sticky before being completely reduced to powder. NMR was very similar to the product from (1) above, but with slightly less isopropanol.

Alternative preparation of GBVH

(a) Ganciclovir was esterified with *N*-benzyloxycarbonyl-L-valine *N*-carboxyanhydride in dimethylformamide in the presence of triethylamine as follows:

A solution of 30 g CBZ-L-valine *N*-carboxyanhydride in 50 mL *N,N*-dimethylformamide was added dropwise to a stirred mixture of the other reagents (11 g triethylamine and 12 g ganciclovir in

35 mL *N,N*-dimethylformamide). The stirring was continued. After 4 hours, TLC indicated a trace of starting material and a slightly less polar compound, otherwise the product was identical to that from the previous preparation of ganciclovir bis(CBZ-L-valinate). Stirring was continued for 16 hours.

The mixture was diluted to a volume of about 500 mL with water. The precipitated solid was collected, washed with water, and air dried. The solid was then dissolved in dichloromethane, in which all solid dissolved, and the solution was filtered. The filtrate was stripped to a viscous glass. The glass was dissolved in ethyl acetate and the solution diluted with toluene. Some of the solid precipitated. The mixture was stripped to a white solid. NMR indicated a trace of ethyl acetate, but, unlike the material prepared in the previous preparation, the NMR spectrum was sharper.

The ganciclovir bis(CBZ-L-valinate) so obtained was purified as follows: a mixture of 30 mL ethyl acetate with 3 g ganciclovir bis(CBZ-L-valinate) was warmed to about 50°C with vigorous stirring. The mixture was allowed to cool with stirring. After about 6 hours the mixture was filtered, and the solid was washed with ethyl acetate and dried in a vacuum oven under a nitrogen bleed, yielding 2.8 grams. TLC of the material indicated the presence of pure ganciclovir bis(CBZ-L-valinate).

(b) The resulting ganciclovir bis(CBZ-L-valinate) was hydrogenated using 0.25 g Pd(OH)₂ on carbon, which was pre-reduced and the hydrogenation carried out as described in preparation (1) previously with the following modifications: HPLC (conditions as given previously) of the reaction mixture after 4 hours of hydrogenation indicated about 3% of the mono(L-valinate) (combined total for the two diastereomers) and 1.5% total of the two impurity peaks seen in the previous experiments. Hydrogenation was continued for 16 hours. The resulting mixture was filtered and the filtrate stripped to yield a viscous oil. The oil was left on the vacuum pump for drying. After complete evacuation, a white foam resulted which was dissolved in 5 mL ethanol. The solution was diluted with 60 mL

ethyl acetate. A gummy solid separated, which slowly hardened with stirring. Stirring was continued further for 16 hours, then the mixture was filtered and the white solid washed with ethyl acetate and dried in a vacuum oven at about 45°C under a nitrogen bleed. Some of the product remained stuck to the walls of the precipitation flask and was somewhat sticky when removed with a spatula. The yield of product was 1.6 g. HPLC data: 95.7% purity with 2.5% ganciclovir mono(L-valinate), 0.6% and 0.8% of the impurities. Karl Fischer analysis indicated 0.77% water. The solid was hygroscopic and lacked luster, indicating a lack of crystallinity. The dried product cake was hard, but somewhat easier to break up than the products from the experiments above. NMR indicated a trace of ethyl acetate and was cleaner than spectra for the products from the previous experiments with all the multiplets well defined.

Conclusion

In my opinion, based on my knowledge and experience of crystallization of organic compounds, in particular acid addition salts, and especially ganciclovir esters, the inability of myself and others to prepare crystalline ganciclovir mono(L-valinate) acetate, and the inability of others to prepare crystalline ganciclovir bis(L-valinate) acetate, I believe that it is not possible to prepare ganciclovir bis(L-valinate) acetate in stable crystalline form because acetic acid is a weak acid with a low dissociation constant ($K_a \sim 1.8 \times 10^{-5}$), so that it does not form strongly bound acid addition salts, which is usually a requirement for the formation of a stable crystalline material.

For this reason, I believe that ganciclovir bis(L-valinate) acetate will not form a stable crystalline compound; but rather will form a compound of variable composition depending on the conditions of preparation, and this compound will be unstable to, for example, gain or loss of solvent of crystallization, so that it may be hygroscopic and/or deliquescent.

For these reasons, and because ganciclovir mono(L-valinate) hydrochloride is readily obtainable in stable crystalline form as set

forth in App. No. 08/453,223, I believe that if a ganciclovir bis(L-valinate) acid addition salt were to be capable of being prepared in stable crystalline form, the hydrochloride would be substantially more likely to be preparable in crystalline form than the corresponding acetate prepared in the Beauchamp patent, and I therefore attempted the preparation of crystalline ganciclovir bis(L-valinate) hydrochloride.

Ganciclovir bis(L-valinate) hydrochloride as prepared is non-crystalline, and repeated attempts to prepare it in crystalline form using conditions calculated to cause crystallization of the material (which conditions lead to ready crystallization of ganciclovir mono(L-valinate) hydrochloride) have been unsuccessful. I believe that it is not possible to prepare ganciclovir bis(L-valinate) hydrochloride in crystalline form without undue experimentation, if at all.

I further declare that all statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment or both under 18 USC 1001, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: April 6, 1999



Charles Dvorak

Heller Ehrman White & McAuliffe
525 University Avenue
Palo Alto, CA 94301-1900
(650) 324-7014